Effect of Androgens on the Growth of Cultured Human Dermal Papilla Cells Derived from Beard and Scalp Hair Follicles

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Androgens stimulate hair growth in some areas, e.g., beard, but may cause regression and baldness on the scalp. The mesenchyme-derived dermal papilla is believed to regulate many aspects of hair growth. It is probable that androgens exert their effect on hair growth via the dermal papilla. In this study the effect of androgens on the growth of cultured dermal papilla cells from beard and non-balding scalp was assessed. Dermal papilla cells from beard hair follicles and non-balding scalp were cultured in vitro in the presence and absence of different concentrations of testosterone or the synthetic, non-metabolizable androgen, mibolerone. Cell growth was reflected by the incorporation of ^{3}H-thymidine.

The presence of either androgen did not significantly alter DNA synthesis at any of the concentrations examined in either type of cell line. These results do not mean that dermal papilla cells do not respond to androgens in vitro, but that the measurement of cell growth is an inappropriate method of assessment. Androgens may well stimulate the synthesis of specific proteins that could influence the hair follicle. *J Invest Dermatol 97:345–348, 1991*

Androgens stimulate human hair growth in many regions such as the beard, axilla, and pubic areas, and have little effect on others such as eyelashes and the occipital regions of the scalp, yet in genetically disposed individuals cause balding on certain areas of the scalp [1].

The mesenchyme-derived dermal papilla is thought to be important in regulating the growth of hair-follicle epithelium [2]. Cultured dermal papilla cells from both rat [3] and human [4] follicles have been reported to display morphologic and behavioral properties that are sufficiently distinct from skin fibroblasts to suggest that they represent a separate "specialized" population of skin fibroblasts.

The complex association of epithelium and mesenchyme in the embryonic development of other tissues such as the prostate [5] and the breast [6] have been well documented. These studies have indicated that steroids may act via the mesenchyme to mediate the differentiation of the epithelium. Autoradiographic localization of ^{3}H-testosterone has shown that in the rat dermal papillae but not hair-follicle epithelium take up androgens [7], suggesting that androgens act on hair follicles via the dermal papillae.

Androgens initiate their action by binding to a specific intracellular receptor present in target cells. We have previously demonstrated that cultured dermal papilla cells contain specific, saturable androgen receptors, and that those cultured from androgen-responsive follicles, i.e., beard, contain significantly higher levels than those from the less androgen-sensitive areas of the scalp [8].

The aims of this study were to determine whether androgens have a direct effect on the proliferation of human dermal papilla cells in culture, and whether dermal papilla cells derived from androgen-sensitive follicles such as the beard and those from the less androgen-responsive non-balding areas of the scalp exhibit different responses to androgens in vitro.

MATERIALS AND METHODS

Cell Culture Dermal papillae were isolated from normal human hair follicles and cultured according to the method of Messenger [9]. Normal skin samples were obtained from a total of 17 patients (14 male and three female) during routine clinical excisions and dermal papillae were microdissected from the base of anagen hair follicles, transferred to 35-mm petri dishes (Falcon Labware), and incubated in medium E199 supplemented with glutamine (2 nmol/ml), penicillin (100U/ml), streptomycin (100 μg/ml) (Flow Laboratories Ltd., UK), and 20% fetal bovine serum (FBS) (Globe farms, UK) for 2–3 weeks. The cell outgrowths were subsequently subcultured into a 25-cm^2 tissue-culture flask (Flow Laboratories) and thereafter subcultured with a split ratio of 1:3.

^{3}H-Thymidine Assay Cells were seeded into 24-well plates (Linbro, Flow Laboratories, UK) at a density of 2×10^4 cells in 1 ml of normal growth medium and incubated at 37°C for 3–4 d until almost confluent. All cells were assayed between passage numbers 3 and 5. The growth medium was removed, and the cells washed with 1 ml of phosphate-buffered saline (PBS) before the addition of 1 ml of serum-free medium. The cells were incubated for 48 h in serum-free medium prior to the assay. Our observations have shown that cultured dermal papilla cells survive well in serum-free medium for this period of time, and that they secrete autocrine growth factors when cultured in serum-free medium [10].

Testosterone or mibolerone was dissolved in absolute ethanol (0.1 M) before diluting in serum-free medium over the physiologic

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Abbreviations:
FBS: fetal bovine serum
PBS: phosphate-buffered saline
TCA: trichloroacetic acid

concentration range of 10^{-5} M to 10^{-10} M for testosterone and 10^{-6} M to 10^{-11} M for mibolerone. The ^{3}H-thymidine uptake by dermal papilla cells treated with each concentration of either androgen was measured in three separate wells for each cell line studied. Three wells were incubated in serum-free medium with the ethanol vehicle alone (0.01%) as controls. The ^{3}H-thymidine uptake of these wells gave an indication of the basal rate of DNA synthesis in each cell line. Cells were also incubated in medium containing 20% FBS as positive controls, and three wells incubated in serum-free medium without ^{3}H-thymidine were also included for cell counting by hemocytometry.

The cells were incubated with androgens or FBS for 24 h, then the medium was removed and replaced with fresh serum-free medium containing the same concentration of androgen plus 0.5 μCi of methyl-^{3}H-thymidine (SA 0.925 TBq/mmol) (Amersham International plc, UK). The cells were incubated for a further 6 h, before removal of the medium and washing with 1 ml PBS (×2). The proteins in each well were precipitated by the addition of a 10% solution of trichloroacetic acid (TCA) for 10 min at 4°C, before digestion in 400 μl of 1 M sodium hydroxide for about 16 h at 37°C. The amount of radioactivity in the cell digests was then estimated using an LKB liquid scintillation spectrophotometer with a counting efficiency of 50%.

Autoradiography Dermal papilla cells (approximately 3×10^4) were seeded into 35-mm petri dishes and grown to confluence in medium E199 with 20% FBS. Cells were washed with PBS (×2) and incubated in serum-free medium for 48 h. The medium was removed and 2 ml of fresh serum-free medium containing ^{3}H-thymidine (0.5 μCi/dish) was added. Control dishes of cells were incubated without ^{3}H-thymidine. After 6 h the incubation was terminated by removing the medium and washing with PBS (×2). Cells were fixed in ice-cold acetic acid : methanol (1 : 3) for 10 min, incubated with 10% TCA for 5 min, and then rinsed in distilled water. The dried cultures were coated in the dishes with Ilford L4 nuclear research emulsion (Ilford Ltd, UK), diluted 1 : 1 with distilled water, drained, and stored for 11 d in a light-tight container at 4°C. The film was developed for 10 min in Kodak D19 developer (Kodak Photographic, UK), rinsed, and fixed for 5 min with acid hardening fixer Kodak F5. The cells were counterstained with Giemsa and examined by microscopy to establish that radiolabeling was restricted to the nuclei.

Statistical Analysis Data are presented as means ± SEM. The difference between the means of two samples was analyzed by Student t test, and by one-way analysis of variance.

RESULTS

Autoradiography The autoradiographs confirmed that ^{3}H-thymidine labeling was confined to the nuclei, indicating that ^{3}H-thymidine uptake did indeed reflect DNA synthesis and, presumably, cell growth.

DNA Synthesis The effects of both serum-free medium and normal growth medium (20% FBS) on the DNA synthesis of dermal papilla cells are given in Fig 1. The presence of serum significantly stimulated the growth of dermal papilla cells ($p < 0.001$).

The effect of a range of concentrations of testosterone (10^{-10} M to 10^{-5} M) in serum-free medium on the rates of DNA synthesis of male beard dermal papilla cells (five lines) and non-balding scalp dermal papilla cells (five lines) is given in Fig 2. The basal rate of ^{3}H-thymidine incorporation into dermal papilla cells was determined by incubating the cells in serum-free medium without testosterone. The level of ^{3}H-thymidine incorporation did not significantly increase above the basal rate at any of the concentrations of testosterone in either type of cell line.

In the event that the testosterone may be rapidly metabolized to less potent androgens the experiment was repeated using the synthetic, non-metabolizable androgen mibolerone (7α, 17α-dimethyl-19-nortestosterone); the results are given in Fig 3. In scalp cells (five lines) the level of ^{3}H-thymidine uptake remained on or below the basal level with each concentration of mibolerone, except at 10^{-11} M, where it was higher, although not significantly. In beard cells (four lines), ^{3}H-thymidine uptake was higher than the basal rate at all concentrations of mibolerone but the difference was not significant. A dose-response relationship was not demonstrated.

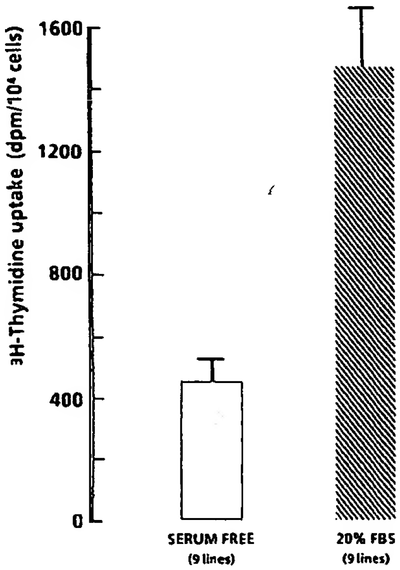

Figure 1. A comparison of the rates of ^{3}H-thymidine incorporation by dermal papilla cells incubated with (□) serum-free E199 medium (nine cell lines) and (▨) normal growth medium, i.e., E199 medium with 20% FBS (nine cell lines). Each was measured in triplicate in each cell line (mean ± SEM).

DISCUSSION

The growth of cells in culture can be assessed by many different methods; a rapid and convenient method using ^{3}H-thymidine is conventional in studies of the proliferative growth of cells in vitro. Other workers have demonstrated that thymidine incorporation used to assess promotion of growth in cultured cells correlates well with other methods, and therefore may be considered a valid indicator of proliferative growth in response to growth-promoting agents [11,12].

The addition of serum significantly increased the rate of ^{3}H-thymidine incorporation into cultured dermal papilla cells, confirming the validity of the assay; autoradiography showed that ^{3}H-thymidine was confined to the nucleus.

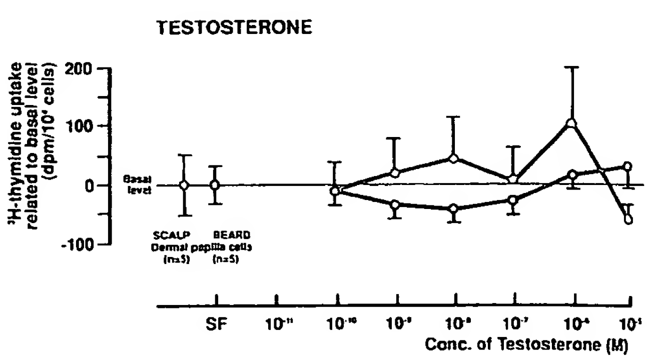

Figure 2. A comparison of the rates of ^{3}H-thymidine incorporation by beard (five cell lines) and non-balding scalp (five cell lines) dermal papilla cells incubated with different concentrations of testosterone in serum-free medium. SF represents the cells incubated in serum-free medium alone to reflect the basal level of ^{3}H-thymidine incorporation. *Points*, means of triplicate measurements on each line (mean ± SEM).

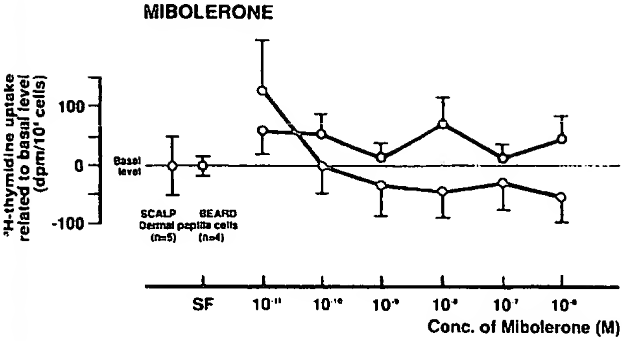

Figure 3. A comparison of the rates of ^{3}H-thymidine incorporation by beard (four cell lines) and non-balding scalp (five cell lines) dermal papilla cells incubated with different concentrations of mibolerone in serum-free medium. SF represents the cells incubated in serum-free medium alone to reflect the basal level of ^{3}H-thymidine incorporation. *Points*, each line measured in triplicate (mean ± SEM).

The growth of many cell lines established from tumors such as prostate and breast, such as the mouse mammary carcinoma Shionogi 115 cell line [13], and the human prostate cancer cell line LNCaP [14], has been shown to be stimulated by androgens in vitro. Steroid hormones have also been shown to have an effect on cultured primary cells; workers have demonstrated that androgens increase the growth of human prostatic epithelial cells in vitro [15] and both epithelial and fibroblast cell lines from the adult canine prostate [16]. However, other workers have failed to demonstrate a growth response in some primary cells such as genital skin fibroblasts [17] and pubic skin fibroblasts [18] that are clearly androgen dependent and contain androgen receptors [19].

In this study, no significant increase in ^{3}H-thymidine incorporation could be demonstrated with a range of concentrations of testosterone by either beard or scalp dermal papilla cells. To eliminate the possibility that this lack of response may have been due to the metabolism of testosterone to weaker androgens over this incubation period, because both beard and scalp dermal papilla cells have been shown to metabolize testosterone [20], the experiment was repeated with mibolerone. However, similar results were obtained.

Other workers have also reported that androgens do not stimulate the growth of cultured scalp dermal papilla cells. Counting studies on cells derived from human scalp follicles showed that the addition of both testosterone and 5α-dihydrotestosterone in fact inhibited cell growth over a period of 12 d [21]. However, at 10^{-6} M and 10^{-7} M the concentrations were considerably higher than the saturation level of the androgen receptor.

We have previously found that cultured dermal papilla cells contain androgen receptors and that beard dermal papilla cells contain significantly higher levels of receptors than non-balding scalp cells [8]. Therefore, the lack of response is not due to the absence of receptors. Our inability to detect stimulation of beard dermal papilla cell growth by androgens in culture concurs with results from other non-tumour cells as discussed above [17,18] and may be because this is an inappropriate end-point for measuring the androgenic responses of primary dermal papilla cell lines. There is little evidence of dermal papilla cell growth even in vivo. The number of cells in the dermal papilla is thought to remain fairly constant during the rat hair growth cycle [22]; the differences in size between the growing (anagen) and resting (telogen) phases are attributed to changes in the extracellular matrix [23]. Although it is clear that androgens stimulate the growth of epithelial hair-follicle cells in the transformation from vellus to terminal, there is no specific evidence that androgens stimulate the growth of dermal papilla cells in vivo. Nevertheless, the size of the dermal papilla is proportional to that of the hair follicle [24,25] so that androgens presumably stimulate an increase in size of the dermal papilla in vivo. Although this could be due to increased production of the extracellular matrix component of the dermal papilla, it seems likely that the number of dermal papilla cells also increases. A slow growth response of dermal papilla cells to androgens in vivo could explain the gradual transformation of follicles from vellus to terminal.

A more appropriate method to assess the responses to androgens might be the initiation or alteration in the levels of the synthesis of specific proteins or mRNA. Other workers have reported that Shionogi 115 cells specifically synthesize several proteins in the presence of testosterone that could be inhibited by the antiandrogen cyproterone acetate [26]. The responsiveness to hormones is also dependent on the composition and spatial configuration of non-diffusible elements of the cellular environment such as matrix, substratum, and other cells [27]. We have already shown that cultured dermal papilla cells produce mitogenic factors [10] and extracellular matrix components [28]. It is possible, therefore, that the mitogenic action of androgens on human hair growth in vivo is indirect and mediated by other growth-promoting factors associated with the extracellular matrix, or with the secretion of specific growth factors. These possibilities are currently under investigation.

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